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DOCUMENT-IDENTIFIER: US 20020081725 A1
TITLE: Culturing pancreatic stem cells having a specified, intermediate stage of development

Detail Description Paragraph (46):
[0056] The present invention provides, inter alia, methods to generate a culture of pancreatic cells that are capable of continued replication, but may be induced to differentiate into more mature cells suitable for therapeutic purpose. Accordingly, some methods of the invention require as an initial step the isolation of cells from the pancreas. Cells harvested from a pancreas are a diverse population that may yield differentiated cells capable of endocrine and exocrine secretion. These differentiated cells express pancreatic endocrine molecules such as insulin, somatostatin, glucagon and other endocrine hormones, as well as pancreatic exocrine molecules such as amylase. Further, a portion of the cultured cell population is capable of replication and expansion in culture. The intermediate cell population of the present invention my arise all or in part by differentiation of facultative stem cells, dedifferentiation of mature endocrine cells, or by transdifferentiation of other pancreatic cell populations. The culture methods described below exploit various extraction and culture conditions to generate various populations of pancreatic cells.

Detail Description Paragraph (60):
[0070] Once the pancreatic cellular material has been harvested and selected for culture, or once a population is confluent and is to be transferred to a new substrate, a population of cells is seeded to a suitable tissue culture container for cultivation. Seeding densities can have an effect on the viability of the pancreatic cells cultured using the disclosed methods, and optimal seeding densities for a particular culture condition may be determined empirically by seeding the cells at a range of different densities and monitoring the resulting cell survival and proliferation rate. A range of seeding densities have been shown to be effective in producing hormone secreting cells in culture. Typically, cell concentrations range from about 10.sup.2 to 10.sup.8 cells per 100 mm culture dish, e.g., 10.sup.2, 10.sup.3, 10.sup.4, 10.sup.5, 10.sup.6, 10.sup.7, or 10.sup.8 cells per 100 mm culture dish, although lower cell concentrations may be employed for cloning procedures. Cell concentration for other culture vessels may be adjusted by computing the relative substrate surface area and/or medium gas exchange surface area for a different culture vessel. For example, a typical 100 mm culture dish has a substrate surface area of 55 square centimeters (see Freshney, supra), and a cell concentration of 10,000 cells per dish corresponds to about 180 cells per square centimeter, while a cell concentration of 100,000 cells per dish corresponds to about 1,800 cells per square centimeter. Cell concentration in terms of culture vessel surface area may be related to cell concentration in terms of media volume by using the appropriate media volume per culture surface area (0.2-0.5 ml/cm.sup.2 are typical ranges for static culture). To determine if a 10 fold expansion has occurred, the cells are removed by enzymatic digestion and counted under microscope in a known volume of fluid. Cells may also be grown on culture surfaces pre-coated with defined extracellular matrix components to encourage growth and differentiation (e.g., fibronectin, Collagen I, Engelbreth-Holm-Swarm matrix, and, preferably, collagen IV or laminin).

Detail Description Paragraph (100):
[0110] A preferred starting material for this procedure is a culture of intermediate stage cells produced by the methods of the present invention, although other cell populations containing PDX-1 positive facultative stem cells may be used. If the

intermediate stage cell populations are simply allowed to grow to confluence, foci or aggregates of cells may appear. Aggregate formation may also be induced by treatment methods specific to cells generated by a particular method of the invention (e.g., intermediate populations generated by shock methodologies may be induced to form aggregates by treatment with collagenase). However, consistent aggregate formation is enhanced if the cells are cultured on a substrate previously used to culture pancreatic cells. Without wishing to be bound by a particular theory, the growth of intermediate stage cells may stimulate the deposition of particular extracellular matrix molecules, or a particular spatial arrangement of the extracellular matrix. If the cells are removed and new cells seeded upon this matrix, the matrix promotes differentiation of the cells into aggregates with morphological and biochemical properties similar to islets in vivo.